Phytochemistry, Vol 28, No 10, pp 2647–2650, 1989.
Printed in Great Britain

0031–9422/89 $3.00 + 0.00

DIHYDROANTHRACENONES FROM *DERMOCYBE SPLENDIDA* AND RELATED FUNGI*

MELVYN GILL, ALBERTO GIMENEZ, AKHIL G. JHINGRAN and ALBIN F. SMRDEL

Department of Organic Chemistry, University of Melbourne, Parkville, Victoria, Australia 3052

(*Received* 14 *December* 1988)

Key Word Index—*Dermocybe splendida*; *Dermocybe* spp., *Cortinarius*; Agaricales, pigments, dihydroanthracenones, biosynthesis

Abstract—(3*S*)-Atrochrysone and (3*S*)-torosachrysone have been isolated from Australian toadstools belonging to the genus *Dermocybe*; labelling experiments show that sodium [2-^{13}C]acetate and [Me-^{13}C]methionine are efficiently incorporated into torosachrysone by intact fruit bodies.

INTRODUCTION

Atrochrysone and its 6-*O*-methyl ether, torosachrysone, occupy what appear to be pivotal positions in the biogenesis of anthraquinone and pre-anthraquinone pigments in fungi [1]. For example, it seems likely that atrochrysone (**1** or its mirror image) is a progenitor of emodin and hence of many other neutral anthraquinones [2]. The (3*R*)-antipode of **1** has been isolated from the toadstools *Cortinarius atrovirens* and *C. odoratus* [3, 4]. Similarly, torosachrysone (**2** or its mirror image) is a logical antecedent to fungal tetrahydroanthraquinones such as austrocortilutein (**3**) and austrocortirubin (**4**) [5], and to a large group of dimeric pre-anthraquinones including the various diastereoisomers of flavomannin dimethyl ether (**5**) [1]. The dihydroanthracenone **2** is a constituent of the seeds [6] and seedlings [7] of the plant *Cassia torosa* and has been isolated from the roots of *C. singueana* [8] but, despite its important role as a precursor to more elaborate fungus pigments, torosachrysone has not, hitherto, been detected in fungi.

We report here the isolation of (3*S*)-torosachrysone (**2**) from *Dermocybe splendida*,† a toadstool from which we have previously characterized tetrahydroanthraquinones of the types **3** and **4** as the principal colouring matters [5, 9] This is the first record of torosachrysone as a fungal metabolite

Much higher concentrations of torosachrysone (**2**) occur in several other toadstools related to *D. splendida*. In one of these we have established the biosynthesis of **2** by feeding experiments, while in a second, torosachrysone occurs together with (3*S*)-atrochrysone (**1**). This is the first report of the (3*S*)-enantiomer (**1**) of atrochrysone as a natural product.

RESULTS AND DISCUSSION

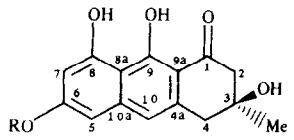
Solvent extraction of fresh fruit bodies of *D. splendida* gave deep red extracts. Preparative TLC effected preliminary separation of an anthraquinone fraction [10] from more abundant, less mobile pre-anthraquinones including, *inter alia*, **3** and **4** [5, 9]. Repeated silica gel chromatography of the pre-anthraquinone fraction followed by gel permeation using Sephadex-LH20 gave the dihydroanthracenone **2** (1.1×10^{-3}%), $[\alpha]_D^{34} + 7.2°$ (dioxan), $[\alpha]_D^{22} - 6°$ (MeOH), R_f 0.35 [toluene–ethyl formate–formic acid (50:49:1)] which showed absorption maxima in the electronic spectrum at 328, 331 and 398 nm consistent with 1,8-dihydroxynaphthalene chromophore conjugated with a carbonyl group.

The molecular formula $C_{16}H_{16}O_5$ for **2** was deduced from mass spectroscopic and analytical data and, together with ^{1}H and ^{13}C NMR data (below), clearly identified this metabolite as torosachrysone (**2**). Thus, the ^{1}H NMR spectrum of **2** in Me_2CO-d_6 at 400 MHz shows a pair of *meta*-coupled aromatic protons (δ6.39 and 6.70, J = 2.2 Hz), hydroxy and aromatic singlets (δ4.04 and 6.95, respectively), C-Me and O-Me resonances (δ1.41 and 3.88, respectively), and signals (δ 9.76 and 16.34) due to chelated hydroxy groups. All of these data are in general agreement with literature values for the plant product [6, 11]. However, in contrast to the literature to date, which records one or both pairs of methylene protons at C-2 and C-4 in **2** as giving rise to broad singlet resonances, higher field strength reveals both signals as clearly discernable AB-quartets. Consequently, the individual components of each of these signals may now be unequivocally assigned (Experimental) in terms of the conformation **6** for the dihydroaromatic ring in **2**.

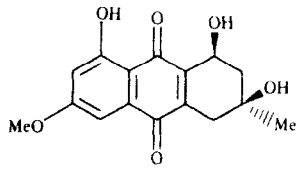
The absolute configuration of torosachrysone isolated from *Cassia singueana* has been established as (3*S*) by exciton chirality experiments involving the 3-*O*-benzoyl derivative of torosachrysone-8,9-di-*O*-methyl ether [8]. Comparison of the specific rotation of the fungal metabolite **2** measured in dioxan with data reported for the

*Part 10 in the series 'Pigments of Fungi'. For Part 9 see *Tetrahedron Letters* (1988) **29**, 2085.

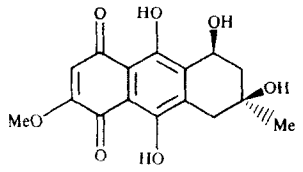
†The fungus described here was originally placed close to *Cortinarius puniceus* Orton and *C. sanguineus* (Wulf ex Fr.) Fr (Watling, R., personal communication) It was not identified by name in earlier publications in this series [5, 9, 10]. It has now been identified as *Dermocybe splendida* Horak by comparison with material collected in New Zealand (Horak, E., personal communication).



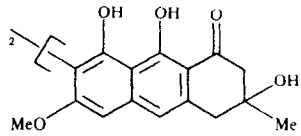
1 R = H
2 R = Me



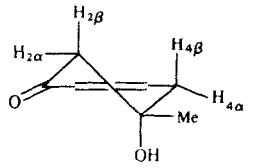
3



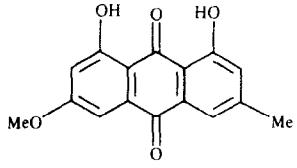
4



5



6



7

plant product $\{[\alpha]_D^{30.5} + 7^\circ$ (dioxan) [6]$\}$ strongly suggests that the dihydroanthracenone **2** from both sources possesses (3*S*)-stereochemistry This suggestion was subsequently confirmed by comparison of the CD spectrum of fungal torosachrysone (Fig. 1) with chiroptical data reported by Steglich and coworkers [12] for the plant product and for the (3*S*)-enantiomer of torosachrysone-8-*O*-methyl ether prepared unambiguously from (−)-quinic acid

This is the first report of (3*S*)-torosachrysone (**2**) as a fungal metabolite and its occurrence in *D splendida* adds credence to our earlier comments regarding the role of dihydroanthracenones in the biosynthesis of pre-anthraquinones such as **3**–**5** in fungi [1, 5, 9]

We have found several species of *Dermocybe* toadstools in S.E Victoria which contain (3*S*)-torosachrysone (**2**) not as a minor pigment but rather as the major colouring matter Two of these species, WAT 20880, and WAT 20881* are discussed further here

WAT 20880 gave a brown ethanolic extract which after purification by silica gel and Sephadex chromatography afforded (3*S*)-torosachrysone (**2**) in a yield of 6×10^{-2}% of the fresh weight of the fungus Traces of physcion (**7**) isolated during preparative TLC and identified from the

*These fungi are placed as new taxa in *Dermocybe* (Watling, R., personal communication) They are referred to here by the accession numbers under which voucher specimens are held in the herbarium of the Royal Botanic Garden, Edinburgh, U K WAT 20881 is thought to be close to *D sphagnetı* (Orton) Mos while WAT 20880 is further from the main *Dermocybe* line

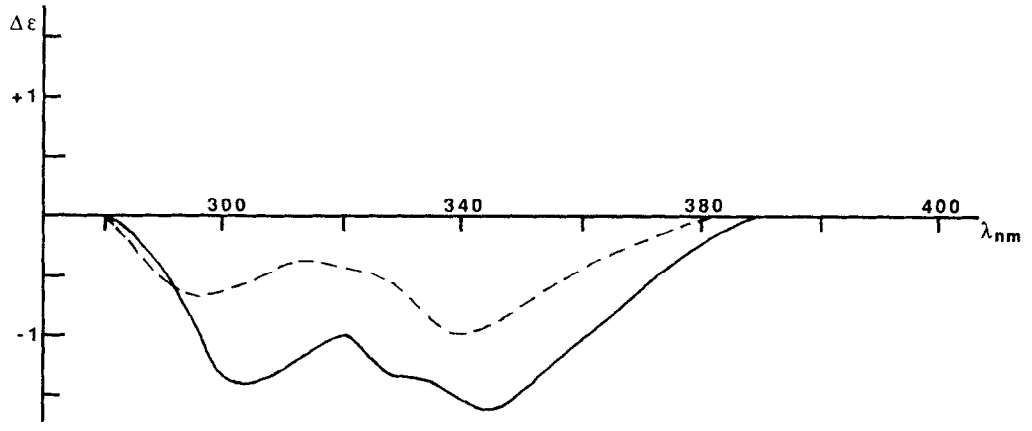
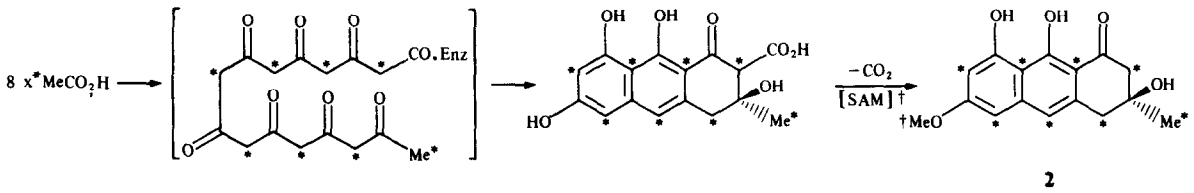


Fig 1 CD spectra of (+)-atrochrysone (**1**) (--) and (−)-torosachrysone (**2**) (- - -) in EtOH



2

Scheme 1 Biosynthesis of torosachrysone (2)

spectroscopic data could have arisen, at least in part, from **2** during the isolation and purification procedure.

In view of the high concentrations of relatively pure torosachrysone in WAT 20880, this organism lent itself to studies of the biogenesis of **2**. Accordingly, a young fruit body growing in its natural habitat was impregnated using a syringe with a total of 100 mg of sodium [2-^{13}C] acetate over a period of three days. After a further four day's growth the toadstool was harvested and torosachrysone (**2**) was isolated in the usual way. The ^{13}C NMR spectrum of **2** isolated from this impregnated toadstool (Table 1) clearly reveals a high incorporation of label at specific sites in **2** which are entirely consistent with the formation of torosachrysone from an octaketide precursor, itself assembled (at least formally) by head-to-tail linkage of acetate units. In a parallel experiment *S*-adenosylmethionine was established as the source of the *O*-methyl carbon atom in **2** by feeding [Me-^{13}C]methionine. High specific incorporation of label‡ into the *O*-methyl group in **2** provides a clear indication that this extra-ketide carbon atom is derived from S-adenosylmethionine. The results of these labelling experiments are summarized in Scheme 1.

Extraction of WAT 20881 gave (3*S*)-torosachrysone (**2**) (8×10^{-2}%) together with a more polar pigment (1.3×10^{-2}%), green-yellow needles, $C_{15}H_{14}O_5$ (mass spectrum and combustion analysis), $[\alpha]_{546}^{22}$ + 8.2° (MeOH), R_f 0.24 [toluene–ethyl formate–formic acid (50:49:1)].

The less mobile pigment was identified as atrochrysone (**1**) from spectroscopic data (Experimental), in particular the similarity between the electronic and NMR spectra and those of torosachrysone (**2**). Once again the individual resonances due to the protons of the C-2 and C-4 methylene groups in the spectrum of **1** are clearly resolved at 400 MHz and may be assigned in terms of the conformation **6** for the dihydroaromatic ring in atrochrysone (**1**).

The (3*S*)-absolute configuration of (+)-atrochrysone (**1**) follows directly from comparison of the CD spectrum (Fig. 1) with that of the (3*S*)-enantiomer **2** of torosachrysone. The stereochemical correspondence between the dihydroanthracenones **1** and **2** might be anticipated from their co-occurrence in WAT 20881 and the close biosynthetic relationship which therefore must exist. Our assignment of (3*S*)-stereochemistry to dextrorotatory atrochrysone complements the work of Steglich *et al.*, who deduced the (3*R*)-configuration for the laevorotatory enantiomer of **1** {$[\alpha]_{546}^{22}$ − 8° (MeOH)} isolated from *Cortinarius odoratus* [3, 12].

‡An enrichment of 15% followed integration of the ^{13}C satellites (J = 143.8 Hz) flanking the 6-OMe resonance at δ3.88 in the ^{1}H NMR spectrum ($CDCl_3$) of **2**.

**For a contrasting suggestion see [13]

Table 1. ^{13}C NMR data for compound **2** isolated from WAT 20880 after impregnation with sodium [2-^{13}C]acetate

C	Chemical shift§ (δ)	Percentage enrichment¶
1	201.4	—
2	50.9	1.0
3	71.0	—
4	43.4	1.1
4a	135.2	—
5	99.2	0.8
6	163.8	—
7	101.0	0.8
8	159.9	—
8a‖	108.0	0.9
9	166.2	—
9a‖	108.0	0.9
10	117.6	0.4
10a	141.0	—
3-Me	28.9	1.1
O-Me	55.6	—

§Data obtained from fully proton-decoupled spectrum in $CDCl_3$. Assignments are consistent with the fully proton-coupled spectrum (Experimental) and have been confirmed by 2D ^{1}H–^{13}C correlation experiments

¶Percentage enrichments were obtained by comparing the intensities of resonances in both the natural abundance and enriched spectra after normalization. Enrichment refers to atom % ^{13}C over and above natural abundance (1.1%)

‖These resonances are resolved (δ_{8a} 108.3 and δ_{9a} 109.3) in Me_2CO-d_6

From our results and those of the Steglich group, it is becoming increasingly apparent that toadstools belonging to *Cortinarius* and its allies are capable of manufacturing, stereospecifically from octaketide precursors, chiral dihydroanthracenones such as atrochrysone with differing absolute stereochemistries**. This conclusion renders the absolute configuration at chiral centres in pre-anthraquinones of the types **3**–**5** a significant question and its determination a serious challenge.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded at 399.65 and 100.40 MHz, respectively in Me_2CO-d_6 (unless stated otherwise) with TMS as int. standard. Mps. uncorr. Prep. TLC used Merck Kieselgel 60 GF_{254} layers (0.1 × 20 × 20 cm) on glass plates. Combustion analyses were performed by the Australian Microanalytical Service, Melbourne, and Chemical and Microanalytical Services, Geelong. Voucher specimens of *Dermocybe splendida* Horak are held in the herbariums of the New South Wales

Department of Agriculture, Biological and Chemical Research Institute, Rydalmere, N S W (accession number DAR 50092) and the Royal Botanic Garden Edinburgh, U K (accession number WAT 18086) Voucher specimens of WAT 20880 and WAT 20881 are also held at Rydalmere under accession numbers DAR 61415 and DAR 61416, respectively Fungi were collected during June 1987 and 1988, feeding experiments were performed during late June 1988

Isolation of **2** *from* D splendida Whole fresh sporophores (455 g) collected near Marysville, Victoria were chopped and immersed overnight in MeOH The red extract was concd under red. pres. and the resulting aq. suspension extracted with EtOAc Evapn of the organic phase and preliminary prep TLC with C_6H_6–HCO_2Et–HCO_2H (50 49 1) gave a mobile zone consisting mostly of anthraquinones [10] and a major polar zone containing pre-anthraquinones including **2** Further silica gel chromatography using the same solvent followed by gel permeation (Sephadex LH-20, MeOH) gave (3*S*)-torosachrysone (**2**) (5 mg, 1 1 × 10 ^{3}% fr wt.).

Isolation of **2** *from WAT* 20880 *and WAT* 20881 Whole fresh sporophores (*ca* 50 g) collected at Kinglake, Victoria were macerated and soaked in EtOH (500 ml) in the dark for 1 hr The brown extract was evapd under red pres and the residue partitioned between EtOAc and H_2O The organic phase was concd and purified by prep TLC with C_6H_6–HCO_2Et–HCO_2H (50 49 1) Filtration through a column of Sephadex LH-20 (in MeOH) gave (3*S*)-torosachrysone (**2**) (33 mg, 6×10^{-2}% from WAT 20880, 44 mg, 8×10^{-2}% from WAT 20881), citrine needles (from MeOH), mp 191–194° (Found C, 67 0, H, 5 6 Calc for $C_{16}H_{16}O_5$ C, 66 7, H, 5 6%), $[\alpha]_D^{34}$ + 7 2° (dioxan, *c* 1 7), $[\alpha]_D^{22}$ −6° (MeOH, *c* 0 65), IR ν_{max}^{KBr} cm^{-1} 1638, 1585, UV λ_{max}^{EtOH} nm (log ε) 228 (4 76), 272 (5 06), 318 (4 23), 331 (4 11), 398 (4 47); ^{1}H NMR δ 1 41 (3H, *s*, 3-Me), 2 78 (1H, *dd*, *J* = 17 0, 1 9 Hz, H-2α), 2 92 (1H, *d*, *J* = 17 0 Hz, H-2β), 3 02 (1H, *dd*, *J* = 16 1, 1 9 Hz, H-4α), 3 10 (1H, *d*, *J* = 16 1 Hz, H-4β), 3 88 (3H, *s*, OMe), 4.04 (1H, *s*, 3-OH), 6 39 (1H, *d*, *J* = 2 2 Hz, H-7), 6 70 (1H, *d*, *J* = 2 2 Hz, H-5), 6 95 (1H, *s*, H-10), 9 76 (1H, *s*, 8-OH), 16 34 (1H, *s*, 9-OH), ^{13}C NMR ($CDCl_3$) δ 201 4 (*t*, *J* = 5 9 Hz, C-1), 166 2 (*d*, *J* = 2 9 Hz, C-9), 163 8 (*m*, C-6), 159 9 (*t*, *J* = 4 4 Hz, C-8), 141 0 (*br s*, C-10a), 135 2 (*t*, *J* = 5 8 Hz, C-4a), 117 6 (*dq*, *J* = 155 5, 5 9 Hz, C-10), 108 0 (*m*, C-8a and C-9a), 101 0 (*ddd*, *J* = 161 3, 5 8, 4 4 Hz, C-7), 99.2 (*dt*, *J* = 161 4, 4 4 Hz, C-5), 71 0 (*m*, C-3), 55 6 (*q*, *J* = 143 8 Hz, OMe), 50 9 (*br t*, *J* = 129 1 Hz, C-2), 43 4 (*br t*, *J* = 129 1 Hz, C-4), 28 9 (*br q*, *J* = 124 7 Hz, 3-Me)

Feeding experiments with WAT 20880 (a) Na [2-^{13}C]Ac A single fruit body was impregnated twice using a syringe (*ca* 10 a m on day 1 and day 3) with an aq soln of Na[2-^{13}C]Ac (250 μl, 2 44 M, 99 atom % ^{13}C) On day 8 (*ca* 4 p m) the toadstool was picked, macerated in EtOH (250 ml) and the torosachrysone (7 mg) isolated as described above The ^{13}C NMR spectrum showed specific incorporation of label as detailed in Table 1

(b) [Me-^{13}C]*Methionine* A single fruit body was impregnated four times using a syringe (*ca* 10 a m on days, 1, 4, 7 and 9) with an aq soln of [Me-^{13}C]methionine (500 μl, 0 53 M, 99 atom % ^{13}C) On day 12 (*ca* 4 p m) the toadstool was picked, macerated in EtOH (250 ml) and the torosachrysone (13 mg) was isolated as before The enrichment in ^{13}C content at the 6-OMe group was measured from the ^{1}H NMR spectrum by integration of the ^{13}C satellites flanking the 6-OMe resonance at δ 3 88

Isolation of **1** *from WAT* 20881 During prep TLC of the extract of WAT 20881 described above a second green zone, more polar than that containing **2**, was obtained Gel permeation (Sephadex LH-20, MeOH) gave (3*S*)-atrochrysone (**1**) (7 2 mg, $1\ 3 \times 10^{-2}$% fr wt), solvated green-yellow needles (from MeOH), mp 234–238 (dec) (Found C, 64 1, H, 5 15 $C_{15}H_{14}O_5$, $\frac{1}{2}$ MeOH requires C, 64 15, H, 5 55%), $[\alpha]_{546}^{22}$ + 8 2° (MeOH, *c* 0 19), IR ν_{max}^{KBr} cm^{-1} 3460, 1643, 1599, UV λ_{max}^{EtOH} nm (log ε) 224 (3 92), 275 (4 25), 320 (3 35), 333sh (3 22), 405 (3.73), ^{1}H NMR δ 1 40 (3H, *s*, 3-Me), 2 77 (1H, *dd*, *J* = 17 4, 2 0 Hz, H-2α), 2 90 (1H, *d*, *J* = 17 4 Hz, H-2β), 2 99 (1H, *dd*,† *J* = 15 9, 2 0 Hz, H-4α), 3 08 (1H, *d*, *J* = 15 9 Hz, H-4β), 4 02 (1H, *s*, 3-OH), 6 37 (1H, *d*, *J* = 2 2 Hz, H-7), 6 58 (1H, *d*, *J* = 2 2 Hz, H-5), 6 83 (1H, *s*, H-10), 9 16 (1H, *s*, 6-OH), 9 84 (1H *s*, 8-OH), 16 52 (1H, *s*, 9-OH), ^{13}C NMR δ 203 8 (C-1), 167 0 (C-9), 162 5 (C-6), 160 9 (C-8), 142 3 (C-10a), 137 8 (C-4a) 117 3 (C-10), 108 9 (C-9a), 107 6 (C-8a), 102 8 (C-7), 101 7 (C-5), 70 7 (C-3), 51 2 (C-2), 43 5 (C-4), 29 5 (3-Me), EIMS (probe) 70eV, *m/z* (rel. int.) 274 0842 $[M]^+$ (100) $C_{15}H_{14}O_5$ requires 274 0841), 256 $[M-H_2O]^+$ (81), 232 (14), 216 (37), 43 (27)

†Clearly resolved only on irradiation of H-10 (δ 6 83)

Acknowledgements—We thank Dr E Horak (ETH, Zurich), Mr T May (Monash University), and Dr R Watling (Royal Botanic Garden, Edinburgh) for help in identifying fungi, Drs R Watling and J Walker (NSW Department of Agriculture, Rydalmere, NSW) for lodging voucher specimens, Miss R A Campbell for some preliminary experiments, and Dr L Gruen (CSIRO Division of Protein Chemistry, Melbourne) for obtaining CD spectra The Australian Research Grants Scheme provided financial support A G and A F S are recipients of Melbourne University and Commonwealth Postgraduate Research Awards, respectively

REFERENCES

1 Gill, M and Steglich, W (1987) In *Prog Chem Org Nat Prods*, Vol 51, pp 125–174 Springer, Vienna
2 Steglich, W (1980) In *Pigments in Plants* (Czygan, F -C, ed) 2nd Edn, p 404 Gustav Fischer, Stuttgart
3 Oertel, B. (1984) Dissertation University of Bonn
4 Steglich, W and Oertel, B (1984) *Sydowia* **37**, 284.
5 Gill, M and Strauch, R J (1985) *Tetrahedron Letters* **26**, 2593
6 Takido, M, Takahashi, S, Masuda, K and Yasukawa, K (1977) *Lloydia* **40**, 191
7 Takahashi, S, Takido, M, Sankawa, U and Shibata, S (1976) *Phytochemistry* **15**, 1295
8 Endo, M and Naoki, H (1980) *Tetrahedron* **36**, 2449
9 Gill, M and Smrdel, A F (1987) *Phytochemistry* **26**, 2999
10 Archard, M A, Gill, M and Strauch, R J (1985) *Phytochemistry* **24**, 2755
11 Kitanaka, S and Takido, M (1984) *Chem. Pharm. Bull.*, **32**, 3436
12 Billen, G, Karl, U, Scholl, T, Stroech, K D and Steglich, W (1988) In *Natural Products Chemistry III*, (Atta-ur-Rahman and LeQuesne, P W, eds), p 305 Springer, Berlin
13 Takahashi, S, Kitanaka, S, Takido, M, Sankawa, U and Shibata, S (1977) *Phytochemistry* **16**, 999